

DESCRIPTIONACTIVE INGREDIENT MATRIX IN THE FORM OF A BIOLOGICALLY RESORBABLE,
POROUS NONWOVEN, METHOD FOR ITS MANUFACTURE AND USE THEREOF

[001] The invention relates to an active ingredient matrix in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form, a method for the manufacture of the active ingredient matrix and the use thereof.

[002] Over the past few years the need for resorbable hemostatics has led to the development of collagen-based products. Collagen sponges have been clinically used in large numbers for many years. As examples of surgical use reference is made to:

- capillary hemorrhages,
- parenchymatous hemorrhages,
- support measures for other hemostatic methods.

[003] Collagen sponges combined with commercially obtainable fibrin adhesion systems are used for arresting diffuse hemorrhages, particularly in parenchymatous organs.

[004] For some years products have been commercially available, which comprise aminoglycoside-filled collagen. These products are lyophilized solutions of dissolved collagen and gentamycin sulphate. The disadvantage of such lyophilized, antibiotic-containing collagen sponges is that the action of the active ingredient rapidly decreases following implantation.

[005] Patent DE 32 12 412 C2 describes a tissue-adherable, collagen wound overlayer. Isolated bovine tendons are homogenized and then the soluble collagen is extracted in citric acid solution or acetic acid pepsin solution. The thus extracted, dissolved collagen is mixed, after dialysis, with corresponding active ingredients such as antifibrinolytics and/or water-soluble antibiotics and then lyophilized.

[006] DE 31 24 981 A1 describes an active ingredient-containing collagen insert for insertion in bones or soft parts. Here the collagen is obtained as a citric acid extract from bovine tendons and this extract is provided with the active ingredient. The extract, comprising dissolved collagen and dissolved antibiotic is lyophilized. If said collagen insert is e.g. introduced into the tibia, it is completely resorbed there within three weeks and during this time antibiotic is released.

[007] European patent EP 360 180 B1 describes a method for the manufacture

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of collagen foams in the form of continuous strips. Here again the collagen is dissolved in that a clearly defined part of the intermolecular bonds is cleaved. Cleaving takes place in such a way that most of the collagen is made water-soluble. The intramolecular bonds of the collagen are not attacked. However, the cleaving can also be controlled in such a way that only a small proportion of intermolecular bonds is cleaved, so that preponderantly higher molecular weight, water-insoluble collagen aggregates are obtained, which are dispersed in water. The aids used for bringing about controlled intermolecular cleaving are not described.

[008] Further methods for the manufacture of collagen sponges by lyophilization of collagen solutions or dispersions have long been known (e.g. EP 209 726, Beutler/Eblinger/Lindner; DE-OS 32 03 957, Eckmayer; US patent 3 157 524, Artandi; DE-OS 33 15 678, Cioca and DE 18 11 290, Chvapil).

[009] There is a need for creating an active ingredient matrix in which the active ingredient action persists over a longer period. In addition, the active ingredient matrix must be easy to manufacture, biologically compatible and able to absorb active ingredients or active ingredient combinations of different types.

[010] The object of the invention is an active ingredient matrix in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form with a long lasting release of active ingredients, containing at least one, homogeneously distributed active ingredient, which is difficultly soluble in water and body fluids.

[011] The active ingredient matrix according to the invention and which is suitable for implantation purposes as an active ingredient-containing collagen, fulfils the following requirements:

[012] The active ingredient or carrier matrix is sterilizable, resorbable, has no permanent tissue reaction, does not disturb wound healing, is easy to apply and offers large-area tissue contact.

[013] Active ingredient release is protracted and complete. It is possible to attain high local tissue levels, linked with a low systemic concentration.

[014] The invention also provides a method permitting the incorporation of active ingredient forms difficultly soluble in water, particularly medicaments, in a collagen suspension. Incorporation takes place in such a way that the homogeneity of active ingredient distribution is ensured.

[015] The object of the invention is a method for the manufacture of a biodegradable active ingredient matrix in the form of an open-cell nonwoven

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of uncrosslinked, resorbable collagen fibrils, particularly of a bovine origin, which is characterized in that cleaned, degreased and dried hide portions are allowed to swell in dilute, organic acids until an elastic material is obtained, the swollen pieces are rinsed several times with aqueous media accompanied by an increase in the pH-value, the swollen pieces are mechanically separated into fibres for forming a suspension of collagen fibrils, the pourable collagen suspension having a pH-value of > 3.5 to < 4.8 is mixed with at least one difficultly soluble active ingredient in finely divided form and homogenized and the active ingredient-containing suspension is then lyophilized to the nonwoven.

[016] Preferably cattle hide is used as the starting material for the active ingredient matrix. For obtaining swellable pieces the hide, preferably after liming and removing the epidermis and subcutis, is comminuted to cube-like pieces, which can then be cleaned, degreased and dried, so that they are in a form suitable for swelling.

[017] The active ingredient matrix according to the invention advantageously has a layer thickness of 0.5 to 15 mm, particularly 2 to 5 mm. In the lower, thin area reference can be made to a nonwoven. In the upper, thick area the active ingredient matrix structure is similar to a sponge and can be referred to as such. The density of the nonwoven is generally between 12 and 180 mg/cm³, particularly between 40 and 80 mg/cm³. The higher the density the longer the resorption time, which also influences the active ingredient delivery time.

[018] Due to manufacture by lyophilization the pore volume of the active ingredient matrix according to the invention can be kept very high and is generally 60 to 80% of the total volume. The average pore size is roughly in the range 20 to 150 μm . The specific surface, measured according to Brunauer, Emmett and Teller (BET) is generally 150 to 350 m²/m² of collagen nonwoven. This also leads to a high, preferred air permeability of 2,500 to 5,000 ml/cm²/min, particularly 2,700 to 3,400 ml/cm²/min, for a layer thickness of 4.2 mm.

[019] Body fluid-difficultly soluble active ingredients are those having in the physiological medium a solubility lower than 10 mg/ml and particular importance and advantage is attached to those active ingredients having a solubility equal to or lower than 1 mg/ml. Active ingredients can have a medicament function, as is generally the case. However, other substances can be used as active ingredients, such as e.g. diagnostics, where it can be just as important that they are slowly released over a longer time period.

[020] The following medicaments are suitable:

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from the class of steroid antibiotics: fusidic acid;
 from the class of sulphonamides: silver sulphadiazine;
 macrolide antibiotics: erythromycin, rifampicin;
 from the class of aminoglycoside antibiotics: clindamicin-palmitate
 clindamicin-palmitate hydrochloride (Sobelin®, Pharmacia and Upjohn) and
 gentamicin-crobecat (E. Merck, Darmstadt, EMD 46/217, EP 173 186 A1, example
 9);
 from the class of glycopeptides: vancomycin
 from the class of quinolones: nalidixic acid, ciprofloxacin.

[021] The active ingredient content of the active ingredient matrix can vary within wide limits. It is generally between 3 and 30 wt.%, based on the total weight of the lyophilized active ingredient matrix and is in particular between 5 and 20 wt.%. The weight per unit area of the active ingredient matrix can vary within wide limits as a function of the layer thickness. It is generally between 1 and 50 mg/cm², particularly between 10 and 20 mg/cm².

[022] Among medicaments with antibiotic characteristics particular preference is given to the aminoglycoside antibiotics clindamicin-palmitate and clindamicin-palmitate hydrochloride, as well as gentamicin-crobecat. The active ingredient matrix according to the invention can also contain several difficultly soluble active ingredients, particularly with different action directions. In a similar manner it is also possible, besides the at least one difficultly soluble active ingredient, to provide one or more active ingredients with less difficult solubility or easy solubility in the active ingredient matrix. Here again it is possible to provide active ingredients with different action directions. Preference is generally given to the provision of active ingredients with the same action direction, but varying rapidity of release. Thus, e.g. in the case of antibiotics, it is possible to combine an antibiotic with retarded release such as gentamicin-crobecat with an antibiotic having the same action direction but a rapid release such as gentamicin-sulphate. This makes it possible to obtain desired, initial high tissue levels with respect to the antibiotic, associated with a long lasting, retarded release of the difficultly soluble antibiotic. The active ingredient matrix according to the invention is consequently eminently suitable for use as an implantable and resorbable depot for active ingredients with a retarded active ingredient delivery, optionally associated with active ingredients having a rapid active ingredient delivery.

[023] During the manufacture of the active ingredient matrix according to the invention it has been found that the handling of the suspension of collagen fibrils, which are largely present in isolated form in the suspension, can be significantly simplified if the suspension has a pH-value of > 3.5 to < 4.8. In this pH-range, the collagen suspension is pourable and makes it possible, following the addition of the active ingredients, to

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homogeneously distribute the same in the suspension, so that they are present in the finished matrix in homogeneous form, embedded between the collagen fibrils. It is fundamentally possible to set the suitable pH-value following the mechanical separation into fibres by adding acid or base. However, it is advantageous for the method and product, to use a preferred procedure in which the acid concentration is so set during the swelling process that following the rinsing of the swollen collagen portions and during the subsequent separation into fibres of the portions into collagen fibrils a suspension is obtained where said pH-range automatically occurs. A pH-range between 4.0 and 4.5 is preferred. As the pH-range is adjustable by choosing the concentration of the organic acid combined with the number of rinsing operations and optionally the rinsing liquid quantity, there are sufficient variation possibilities for obtaining the desired result. Normally at least two rinsing operations and in particular at least five such operations are performed. In a rinsing cycle the rinsing water is removed after each rinsing operation. Generally rinsing or washing takes place with demineralized water. If the active ingredient is added in aqueous medium, e.g. in a suspension, then the latter preferably has roughly the same pH-value in order to avoid pH-shifts as a result of active ingredient addition. Due to the fact that demineralized water is used during rinsing or washing and there is no need for pH-correction after rinsing, the introduction of salts into the active ingredient matrix is avoided, so that the latter is substantially salt-free, which is desirable in many cases.

[024] Swelling can be carried out for a period of 5 to 60 hours and in particular 6 to 48 hours. The swelling period is dependent on the acid concentration and the origin of the collagen material. The organic acid concentration during the swelling process is normally 0.01 to 2 N, particularly 0.05 to 0.5 N and in general is 0.1 N. A suitable organic acid is acetic acid. Other organic acids can be used and preference is given to those having a biological compatibility. Volatile acids are particularly preferred, because they are removed during lyophilization.

[025] As a result of swelling the hide portions which, as stated hereinbefore, are in particular of a bovine origin, are swollen to 3 to 10 times, particularly 4 to 8 times their weight. The size of the precleaned, dry hide portions is advantageously chosen in such a way that after swelling pieces with a diameter of approximately 1 cm are obtained. This size is advantageous for handling and subsequent separation into fibres. Following on to the swelling operation and after removing the wash water, the swollen collagen granulate is received in demineralized water in order to prepare it for the subsequent separation into fibres. The water quantity is preferably chosen in such a way that a 0.1 to 10% mixture, based on the dry collagen material, is obtained. Separation into fibres preferably takes place by dispersion, accompanied by stirring and the collagen structure is broken up

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whilst producing isolated collagen fibrils. After adding the at least one difficultly soluble active ingredient and optionally further, less difficultly soluble active ingredients to the suspension and further homogenization, the suspension is ready for lyophilization, without any other intermediate treatment being necessary.

[026] The invention is further illustrated hereinafter by the description of preferred embodiments, which also reveal the more detailed contexts, together with a performance example.

Swelling stage

[027] The cleaned cattle hide collagen is now swollen in a first working up stage with dilute acid, preferably 0.1 N acetic acid, over a period of 6 to 48 hours, preferably 16 hours. As a result of acid swelling from the hard, brittle cattle hide collagen is obtained an elastic material from which in a subsequent stage with the aid of a mechanical process (cutting process) the collagen fibrils can be isolated.

[028] The acetic acid concentration during the swelling stage is chosen at 0.1 N in such a way that the collagen suspension to be produced in the second stage has a pH-value of 4.0 to 4.5. If the pH-value of the collagen suspension to be prepared in the second stage is above 4.8, the collagen fibres are precipitated from the suspension and there is no longer a homogeneous solution. If the pH-value is below 3.5, the collagen mass is viscous and can no longer be processed.

Rinsing stage

[029] Using two test mixtures the influence of the rinsing cycles on the pH-value or acetic acid concentration in the rinsing water is tested.

[030] The pH-value is determined potentiometrically. The acetic acid concentration in the rinsing water is determined enzymatically.

[031] The following measured values are obtained:

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Table 1: pH-value and acetic acid concentration for the rinsing solutions

Solution	pH-value test 1	pH-value test 2	Conc. acetic acid (mg/l) test 1	Conc. acetic acid (mg/l) test 2
0.1 N acetic acid mixture	2.85	2.82	6.37	6.40
16 h swelling with collagen	3.78	3.69	5.35	5.47
1st rinsing	3.90	3.94	0.36	0.33
2nd rinsing	3.85	3.85	0.16	0.12
3rd rinsing	3.88	3.95	0.12	0.07
4th rinsing	3.93	4.00	0.13	0.05
5th rinsing	3.98	4.05	0.08	0.05

[032] As can be gathered from the table a 5 times rinsing is sufficient for giving a pH-value of approximately 4.0 in the rinsing solution.

[033] As has already been stated, the acetic acid concentration of 0.1 N in the swelling process was chosen so that during the preparation of the collagen suspension a pH-value of 4.0 to 4.5 occurs. During the swelling process the collagen granulate weight has risen by a factor of 4 to 8 by a corresponding water absorption. It is now in the form of a soft, flexible, elastic material of dimensions 1 x 1 x 1 cm.

Mechanical comminution

[034] To the swollen collagen granulate is added sufficient water to provide a 0.1 to 10% suspension, which is homogenized in 2 to 15 minutes in a high power dispersing machine at 400 to 1200 r.p.m. As a result of the mechanical working up process a suspended, wide-mesh fibre braid is obtained, which no longer has any points in common with the in vivo arrangement of collagen fibres in the corium. The in vivo strong collagen fibres braiding through in all directions are broken up and are obtained in the form of shorter fragments.

[035] It has been found that a change in the pH-value of the collagen suspension by adding 0.1 N caustic soda solution or 0.1 N hydrochloric acid

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leads to the following modifications in the consistency of the suspension:

Set pH-value of collagen suspension	Collagen suspension consistency
3.07	Gel, semifluid
3.60	Gel, semifluid
4.03	Pourable suspension
4.30	Pourable suspension *
4.52	Pourable suspension
5.02	Very highly fluid, fibril aggregation
5.55	Long collagen fibrils, separation of fibres and liquids

* Suspension prepared without acid and solution addition.

[036] The following observations were made at the different pH-values set on the collagen suspension:

[037] Below a pH-value of 3.6 the collagen suspension is semifluid. However, there is no agglomeration of fibres to fibre bundles. There is no separation of fibres and liquid.

[038] If to a collagen suspension of pH-value 3.6 is added the corresponding caustic soda solution quantity in order to obtain a pH-value of 4.3, then from the viscous gel once again a highly fluid suspension is obtained. On further increasing the pH-value (5.0), there is a separation of fibres and liquid and the individual fibres agglomerate to fibre bundles.

[039] The tests proved that in the preferred pH-range of 3.8 to 4.5 there is always a pourable collagen suspension, which can be further processed without difficulty.

Homogenization

[040] To the collagen suspension is now added the corresponding quantity of the difficultly soluble medicament.

[041] The term difficultly soluble medicaments here in particular covers active ingredients having in the physiological medium a solubility of < 1 mg/ml.

[042] After adding the difficultly soluble medicament the active ingredient-containing suspension is homogenized for 5 to 30 minutes accompanied by

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stirring with an electric stirrer at 20 to 300 r.p.m. The active ingredients are introduced in the form of an aqueous slurry or in power form.

[043] Lyophilization of pharmaceutical products has been carried out for many years with the main aim of in particular bringing unstable medicaments into dry, storable forms from which it is possible to produce sterile medicaments.

[044] The most important stage in lyophilization is the freezing phase. In this phase the crystal lattice is created from which the following sublimation takes place. As the collagen suspension is not a homogeneous solution, but instead a multisubstance system with solid ingredients, of the conventionally performed tests for determining the freezing parameters such as the determination of the eutectic point, the determination of the collapse temperature and DSC measurements in the low temperature range, only the eutectic temperature was determined. Tests on several collagen suspensions from several collagen granulate batches gave a freezing point reduction to a range -2 to -4°C for a collagen suspension with 2 wt.% collagen. The eutectic temperature for the combination of collagen suspension and difficultly soluble medicament is preferably separately determined for each preparation. The data obtained during the determination of the eutectic point form the basis for the control of lyophilization. The prerequisite for a correct lyophilization for the active ingredient-containing collagen suspensions is that there is a clear drop below the eutectic temperature.

[045] Changes to the pH-value of the collagen suspension by adding caustic soda solution or hydrochloric acid leads to the following changes in the physical stability of the lyophilized nonwoven or the time necessary for complete wettability.

Table 3: Influence of pH-value of collagen suspension on the physical stability of the lyophilized collagen matrix and the time for complete wetting after immersion in water.

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Set suspension pH-value	Complete wettability time (s)	Physical stability of lyophilized matrix
2.98	30.2	good
3.40	42.7	good
3.87	27.6	good
4.30	10.8	good
4.45	12.6	good
4.90	7.0	bad *
5.34	6.1	bad *

* Bad stability is understood to mean that the lyophilized fleece breaks down into individual fibre aggregates after placing in water.

[046] The tests show that collagen nonwovens with a good "wet" stability are always obtained from collagen suspensions in the pH-range 3.8 to 4.5.

[047] The following further conclusions are made:

- pH-values < 3.8 give a slower wettability than nonwovens with pH-values > 3.8;
- for set pH-values 4.5, there is a pronounced decrease in the stability of the nonwovens produced;
- the optimum pH-value setting for the collagen suspension is between 3.8 and 4.5;
- if the collagen suspension pH-value is above 4.8, the collagen fibres are precipitated from the collagen suspension and there is no longer a homogeneous solution.

[048] The active ingredient-containing collagen matrix obtained from cattle hide can give as a result of multistage mechanical and chemical working up processes a wide-mesh fibre braid (light microscopic result), which no longer has any points in common with the in vivo arrangement of collagen fibres in the corium. The in vivo-strong collagen fibre bundles braiding through in all directions are broken up and are obtained as shorter fragments, so that a system is obtained with wide interfibrillar gaps. During lyophilization a coarse meshed network is obtained which, considered spatially, can be compared with a natural sponge. A very large, inner surface increase is obtained, which is formed by collagen fibres.

[049] Electron microscopic section preparations reveal a pattern of collagen fibrils in the form of varyingly thick bundles, which run in all directions in space. Thus, there are longitudinal, transverse and tangential sections of collagen fibrils. Longitudinal fibrils have a typical transverse

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[050] In each case 1 cm² pieces are cut from two different collagen implants (matrix with clindamycin-palmitate and matrix with clindamycin-palmitate hydrochloride). The pieces are weighed and the weight recorded. Subsequently the active ingredient-containing implants are placed in test tubes with in each case 5 ml of 0.066 M phosphate buffer (pH = 7.4) or human serum and eluted in the water bath at 37°C. After 24 hours the implant is removed and placed in fresh buffer solution or fresh serum. The total elution time is 10 days for a 24 hour buffer change. The biological measure used for the antibiotic concentration is the inhibiting action (determining the inhibition halo diameter) on the test organism *Staphylococcus aureus* ATCC 6538 and *Micrococcus luteus* DSM 348. The inhibiting action of the sample is compared with the inhibiting action of stepped doses of a standard (here clindamycin hydrochloride). From identically large inhibition halos for samples and standards, it can be concluded that there are identical antibiotic concentrations.

[051] The delivery of the antibiotics from the different implants can be gathered from table 4.

Table 4: Active ingredient delivery of clindamicin-palmitate as a function of the elution time

Days	Clindamicin-palmitate in phosphate buffer (µg implant)	Clindamicin-palmitate hydrochloride in phosphate buffer (µg implant)	Clindamicin-palmitate in serum (µg implant)
1	18.3	16.0	8.0
2	46.1	23.8	21.2
3	46.1	46.1	34.7
4	52.7	35.4	29.1
5	46.1	46.1	36.1
6	60.1	46.1	37.8
7	46.1	35.4	22.1
8	18.3	20.8	16.5
9	12.3	7.2	10.8
10	12.3	5.5	8.1

[052] The table clearly reveals that the antibiotic delivery from both implants is still not ended after 10 days. The antibiotic quantity to be delivered is approximately the same per unit of time for clindamicin-palmitate ester and free palmitate ester.

[053] To check whether antibiotic residues have been left on the carrier material, the implants were placed after a 10 day elution period on an agar surface contaminated with *Staphylococcus aureus* or *Micrococcus luteus* and incubated at 37°C. After this time period antibiotic could still be detected in the collagen sponges.

[054] If there is a demand for implants able to protect a wound area over a 5 to 15 day period, the materials described here are suitable for ensuring this protection. There is a combination of matrix and active ingredient, which releases the active ingredient in delayed form and in suitable concentrations over a 5 to 15 day period. In addition, up to complete resorption the matrix is protected against bacterial colonization by the adhering biotic. In the investigation of the active ingredients clindamicin-palmitate and clindamicin-palmitate hydrochloride it was surprisingly found that contrary to the results published in the literature (Antimicrobial Chemotherapy, published by David Green Wood, 3rd edition, Oxford University Press, 1995), clindamicin esterified with palmitic acid also reveals an antibiotic activity. In the case of elution in the aqueous buffer system, surprisingly antibiotic activity was detected over a period of at least 11 days. The esterification of the OH-group of clindamicin with palmitic acid admittedly drastically reduces clindamicin solubility (see table 5), but does

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Table 5: Irregular solubility behaviour of the palmitate ester of lincomycin and clindamicin*

pH	Lincomycin-palmitate hydrochloride (mg/ml)	pH	Clindamycin-palmitate hydrochloride (mg/ml)
2.3	0.124	3.7	53.2
-	-	5.8	< 0.001
7.7	0.0249	7.4	< 0.002

Performance example

[055] After swelling, 1823.1 g of collagen granulate (water content 15.0%, average granulate size 4 to 6 mm) are rinsed in 36 l of 0.1 N acetic acid for 16 hours 5 times with 5 l of water for injection purposes. After the fifth rinsing the pH-value of the rinsing solution must be > 4.0, otherwise further rinsing is needed.

[056] Then topping up with water for injection purposes takes place to a total weight of 90.0 kg, whilst taking account of the subsequently to be added quantity of difficultly soluble medicament. The above suspension is homogenized for 2 minutes at 680 r.p.m. in a high power dispersing machine. The pH-value of the homogenized mass is checked.

[057] To the collagen suspension are now added 224.48 g of clindamicin-palmitate hydrochloride (corresponding to 136.26 g of clindamicin base) suspended in 5 l of 0.1 N acetate buffer at pH 3.6 to 3.8. The active ingredient-containing suspension is again homogenized for 15 minutes with an electric stirrer at 150 r.p.m. The total mass of the active ingredient-containing suspension is now 90.0 kg.

4. Lyophilization

[058] For determining the pouring weight on the lyophilization dishes (size 45.6 x 45.6 cm), the necessary active ingredient-containing collagen suspension quantity per nonwoven is related to the available surface area per lyophilization dish.

[059] The plat size of the collagen nonwovens after lyophilization is 43.5 cm x 43.5 cm = 1892.15 cm². An active ingredient-containing collagen nonwoven of dimensions 5 x 8 cm = 40.0 cm² contains 35 mg of clindamicin and 400 mg of anhydrous collagen.

$$\text{Clindamicin-base/dish} = \frac{1892.25 \text{ cm}^2}{40 \text{ cm}^2} \times 0.035 \text{ g} = 1.6557 \text{ g}$$

Thus: $\frac{1.6557 \text{ g}}{136.26 \text{ g}} \times 90000.0 \text{ g} = 1093.6 \text{ g}$ collagen-suspension are to be poured

on each lyophilization dish.

[060] The filled lyophilization dishes are introduced into the lyophilization plant. The following details apply to the lyophilization process:

charging the plant:	+ 7°C
product temperature on freezing:	+ 7°C -45°C
chamber internal pressure on freezing:	10 ³ mbar
product temperature during main drying:	- 45°C +38°C
chamber internal pressure during main drying:	0.9 mbar
product temperature during redrying:	+ 38°C +21°C
chamber internal pressure on redrying:	0.03 mbar

5. Sterilization

[061] Sterilization takes place by radiation sterilization with 25 kGy or ethylene oxide.

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